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April 20, 2006

Mr. Stephen L. Johnson, Administrator
US Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116

Attn: Chemical Right-to-Know Program

RE: **Revisions/Updates to the Test Plan and Robust Summaries for Hexaoxatricosane (CAS No. 143-29-3)**

This letter is submitted by the Rohm and Haas Company in response to comments received from the U.S. Environmental Protection Agency (EPA) in a letter dated February 23, 2006, and posted to the High Production Volume (HPV) Challenge Program website on March 1, 2006. These comments were based on the EPA's review of the Test Plan and Robust Summaries for Hexaoxatricosane (CAS No. 143-29-3).

Rohm and Haas would like to thank the EPA for their careful review of those documents. We have provided responses to each of the EPA's comments in tables attached to this letter. Furthermore, we have also modified our Test Plan and Robust Summaries to reflect some of the EPA's comments and our responses. These changes are also detailed in the attached tables.

The modified Test Plan (.doc and .pdf) and Robust Summaries (.exp, .rtf and .pdf), as well as this cover memo with attached tables (.pdf) are being transmitted electronically via e-mail.

Best regards,

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EPA COMMENT(-)	ROHM AND HAAS COMPANY RESPONSE(S)
Melting point. The submitter did not determine the melting point because "the test substance is a liquid below 0 °C". This qualitative statement is inadequate. Estimated values under 0 °C, are adequate for the purposes of the HPV Challenge Program. The submission needs to include the estimated value.	Hexaoxatricosane is a liquid at 0°C. Section 6.1 of the EPA document, "Determining the Adequacy of Existing Data", states that "it may not be necessary to measure melting points for liquids with estimated melting points of less than 0°C." Previous draft guidance had stated that reporting a melting point of "<0°C is sufficient." In light of the more recent guidance, **Rohm and Haas will modify its Test Plan and Robust Summary to reflect internal non-GLP data indicating a freezing point/pour point for hexaoxatricosane of -44°C.** It is worth noting that EPIWIN v. 3.12 predicts a melting point of 122.54°C for hexaoxatricosane, indicating that other EPIWIN-predicted values for the compound may not be adequate.

EPA COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
Partition coefficient. The submitter provided a measured log Kow value of 6.2. This value appears anomalous for this class of chemicals (polyglycol ethers); EPA found a calculated log Kow of 1.67 (EPIWIN v. 3.12). Because these values differ significantly, and for other reasons discussed below under Water Solubility, the submitter needs to address the apparent discrepancy. The robust summary for this endpoint did not state whether any of the reference compounds were structurally related to the test substance, as recommended in the cited guideline; the method used may not be appropriate for this type of substance.	Initially, the shake flask method (OPPTS 830.7550) was planned, but due to concerns regarding the potential formation of an emulsion [see discussion regarding water solubility] the HPLC chromatographic estimation method (OPPTS 830.7570) was used. Calibration standards were prepared using selected reference substances specified in the Guideline. The reference standards included thiocarbamide, aniline, benzene, toluene, naphthalene, phenantbrene, 2,6-diphenylpryidine, triphenylamine and DDT. This approach is listed in Section 4(ii) in the guideline. The reference standards listed in the guideline did not contain structural analogs to the test substance, nor were any analogs with known log P values readily available. Standards were therefore chosen with log P values designed to bracket the estimated Log P of the test material [1.65, EPIWIN v 3.121 and to create the regression line plotting Log k versus Log P. The value obtained was considerably different than the estimation value but did fall within the standard range. When the results of the water solubility test [see water solubility discussion] were taken into consideration, the high log P_{ow} value was considered appropriate. Therefore, the experimental value derived according to the guideline was determined to be the preferred value. **This test is considered valid. The Robust Summary was modified to incorporate the identity of the reference standards, and their retention times in the HPLC assay. The retention time of Hexaoxatricosane was also included.**

EPA COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
Water solubility. The submitter provided a semi-quantitative measured water solubility value of <0.0001 mg/L at 20 °C. However, in the acute toxicity to invertebrates robust summary (section 4.1) a 92.3 mg/L test solution was clear and colorless with no visible precipitate or surface film throughout the study. In addition, the measured values for water solubility (< 1 µg/L) and log Kow (6.2) do not agree with the expected characteristics of this class of chemicals (polyglycol ethers). A structurally analogous polyglycol, BB300 (butyl[OCH2CH2]n-O-butyl where n =3 to 5) has a water solubility of 2 wt% (20 mg/mL). The deviations from expected values and from observed behavior (solubility in invertebrate study) send a strong signal of problems that the submitter should have addressed. A common factor in the solubility and log Kow measurements is the initial absorption of the test substance onto a silica or treated silica substrate, which may account in some way for the apparently anomalous results in the two tests. The submitter needs to address the conflicting water solubility and partition coefficient issues.	Initially, the shake flask method OPPTS 830.7840 was used to determine the water solubility. A 1 -mL aliquot of the test substance and 30 mL of water were added to a centrifuge tube (dose rate of approximately 30 g/L). The tube was capped and shaken (platform shaker) for approximately 2 hours, then centrifuged. Following centrifugation, the sample was observed to have two layers, with the larger aqueous layer being cloudy. Due to the cloudiness after centrifugation, *it was believed that the test substance was forming an emulsion in the water* (Note: solutions at concentrations >200 mg/L, prepared for ecotoxicology testing, were cloudy at test initiation and throughout the duration of the study.). Based on the biphased nature of the prepared water solubility sample it was determined that the guideline water solubility shake flask method was not appropriate for the test substance. It was assumed that the guideline column elution method would offer a better option for determining the water solubility. The column elution method *was* performed *using ultrapure* water as follows: A 0.32 g of test substance was coated on 60.0 g of sand. Aliquots of the sand were extracted and verified analytically to contain the test substance. The system was equilibrated overnight at a flow rate of 0.4 mL/min. During the equilibration period all column eluent was discarded. Over the next two days, fifty 50-minute eluent fractions were collected in 1 l-dram glass vials (0.4 mL/min x 50 minutes = 20 mL per fraction). The flow rate of the water through the column was then lowered to 0.2 mL/min. After equilibrating for -7 hours, forty-two 100-minute eluent fractions were collected in the same manner as described for the 0.4 mL/min samples (0.2 mL/min x 100 minutes = *20* mL per fraction). The samples were observed to be clear and colorless with no particulate/colloidal matter. The eluent fractions from each flow rate were combined such that five vials were combined to form one sample (i.e., fractions 1A, 1B, 1C, 1D, and 1E were combined to form sample 1). The contents of each flask were transferred to individual 250-mL separatory funnels. To each flask was added 25 mL of ethyl acetate. The flasks were rinsed, and the ethyl acetate was transferred to the individual separatory funnels containing the method validation samples. Each sample was extracted with a total of three 25-mL aliquots of ethyl

acetate. After each extraction, the layers were allowed to separate. The aqueous (lower) layers were transferred back into the 100-mL flasks. The organic (upper) layers were transferred through sodium sulfate and into individual 250-mL flat-bottomed flasks. The aqueous layers were then transferred back to separatory funnels. After the final extraction, the sodium sulfate was rinsed with 25 mL of ethyl acetate. This rinse volume was pooled with the three 25-mL extract volumes in 250-mL flat-bottomed flasks.

The extracts were concentrated to approximately 2 mL using rotoevaporation. The rotoevaporated samples were transferred to 15-mL culture tubes. The 250-mL flat-bottomed flasks were each rinsed with two 5-mL aliquots of ethyl acetate. These rinse volumes were combined with the concentrated samples in the respective culture tubes. The solvent in each culture tube was evaporated to dryness under a gentle stream of nitrogen. A 2-mL portion of methanol was added to each culture tube for the samples. The tubes were capped, shaken by hand, and vortexed. The samples were analyzed by gas chromatography (GC).

Analysis showed varying results, but the results were consistently at or below the concentration of the lowest standard.

For both the shake flask and column elution methods the pertinent guidelines were strictly followed. All indications from these studies are that the test substance was not soluble in water (i.e., less than 0.1 mg/L), but that the test ***substance does form an emulsion that is not separated by guideline methods.*** This result is consistent with the experimentally-determined log P_{ow} value (i.e., low water solubility, high partition coefficient).

Rohm and Haas considers these data to be valid. The Robust Summaries for this section have been modified to reflect the fact that the solubility test was performed in ultrapure water.

The apparent discrepancy between these results and the Hexaoxatricosane concentrations reported in the ecotoxicological tests is addressed under the Ecological Effects section.

EPA COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
Stability in water. The submitter argues that the compound has no water-sensitive groups and that low water solubility precludes this test. However, as already noted, solubility in the ecotoxicity testing was apparently not an issue. Furthermore, hexaoxatricosane contains an acetal group, which is typically susceptible to hydrolysis at acidic pH. Therefore, the submitter needs to supply measured stability-in-water data according to OECD TG 111.	Since the solubility was reported as <0.1 mg/L, samples could not be prepared at less than half solubility and thus the hydrolysis study could not be performed in accordance with the guideline as written. As indicated in the ecotoxicity discussion, Rohm and Haas contends that the "solubility" seen in the ecotoxicity testing was actually a microcolloidal suspension or an ultrafine emulsion (see discussion of ecotoxicity data in Ecological Effects section). **Because Rohm and Haas believes the reported water solubility data to be valid, it remains our position that Hexaoxatricosane's low water solubility precludes a stability-in-water test.** **The Test Plan and Robust Summaries were changed to reflect the possibility of limited hydrolysis.**

EPA COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
Biodegradation. Although the results, "Not readily biodegradable" are adequate for the purposes of the HPV Challenge program, the submitter stated that "The abiotic sterile control system indicated that CO2 production in the test substance systems may be attributed to biodegradation since abiotic degradation was 6.0 % ThCO2 by day 29." However, 6% ThCO2 in an abiotic control is too high and suggests possible problems with the technique such as: (1) the inhibitor did not work as intended (concentration too low, wrong inhibitor, etc.); (2) that the air used in the test was not CO2-free air as required by the method; or (3) the air was not adequately scrubbed free of CO2 during the test. The submitter needs to address the discrepancy.	The potential for degradation of a substance by abiotic processes (i.e., chemical versus biological degradation) differs for each test substance. Some may undergo a considerable amount of degradation by abiotic processes, so a result of 6% $ThCO_2$ is plausible. Microbial evaluation was performed on the abiotic control samples in question, and showed no colony forming units (CFU) in the plated samples (i.e., no microbial contamination). Therefore, sterility of these samples was verified. The degradation results indicate that a minimal amount (6.0% $ThCO_2$) of the proposed degradation was from abiotic processes. The majority of the test substance degradation measured was therefore due to the microbes in the samples. Note: The inclusion of an abiotic control is not an absolute requirement of the test guideline, but can be included if abiotic processes may be a route of degradation. The air supplied to the systems flowed through traps containing dririte and then ascarite prior to reaching a manifold that was used to control the flow rate. Once out of the manifold, the air passed through a trap containing 5N KOH, then a trap containing water to humidify the air before passing through the samples. With two sets of traps designed to remove CO_2, the air was scrubbed of CO_2. The process was verified during the study by the control systems (not to be confused with the abiotic control sample). The goal of the control systems was to differentiate the background CO_2 values from the endogenous CO_2 evolution from the microbial inoculum or that introduced by not adequately scrubbing the air of CO_2 before entering the system. The total mg CO_2 evolved from the controls (15.6 and 12.6 mg CO_2/flask) was within the limits indicated in the guideline (<40 mg CO_2/L or <120 mg CO_2/flask). **No changes were made** to **the Test and Plan and Robust Summaries.**

EPA COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
Ecological Effects (fish. invertebrates. and algae) EPA reserves judgement on the adequacy of ecological effects data pending resolution of water solubility, log Kow and stability-in-water issues.	The *Daphnia magna* $EC_{50,}$ based upon mean measured concentrations, was 87 mg/L. The test solutions were prepared in laboratory freshwater that was *biologically aged* (held in a tank containing aquatic organisms), passed through a sediment filter and W irradiated prior to use. In addition to a control, nominal test substance concentrations evaluated were 25, 50, 100, 200,400, and 800 mg/L, and all test substance concentrations were greater than the reported water solubility (see discussion of water solubility data). The observations of the test solutions described the 200,400, and 800 mg/L treatments as cloudy at test initiation and throughout the duration of the study, which suggests that the organisms were exposed to a colloidal suspension or an ultrafine emulsion. There was also a surface film noted initially in the 400 and 800 mg/L treatments, which was persistent in the highest treatment level. The treatments were verified from analytical evaluation of a single sample collected from each treatment level at 0 and 48 hours of the test. Since the treatment solutions were prepared in a single vessel and distributed into replicate test chambers, only a single analysis is needed as per OPPTS 850.1000 (k)(l)(i). The samples were not centrifuged *as* suggested for samples that may be tested above the solubility of the test substance as described by OPPTS 850.1000 (i)(4). The treatment sample analytical recoveries at initiation were 102, 173, 99, 98, 88, and 91% of the nominal concentrations. After 48 hours of testing, the recoveries of the treatment samples were 90, 89, 85, 92, 97, and 84% of the nominal. Contamination either during the sample collection or processing was considered the likely cause of the high recoveries in the 50 mg/L treatment at test initiation and does not represent the actual exposure to the organisms, which is supported by the 48-hour recovery of 89% from this treatment. There were no residues above the MQL (i.e., 5.85 mg/L) in the control solution samples. The treatment immobility was 0, 20, 25, 0, 70, 100, and 100 % in the control, 25, 50, 100, 200, 400, and 800 mg/L, treatment respectively. There were sublethal effects noted in the 25 (15% of surviving organisms effected), 100 (5% of surviving organisms effected), and in the 200 mg/L treatments (100% of surviving organisms effected), respectively. In addition to the effects seen at high doses, 400 and 800 mg/L, immobilization and sublethal effects were observed at the 25, 50, and 100 mg/L test concentrations that would have been considered to be either in solution [*unlikely however since the test concentrations were above the measured water solubility of the test substance] or* as a microcolloidal suspension or ultrafine emulsion based upon the lack cloudiness or surface

films. These data, in conjunction with the mean measured concentrations indicate that the organisms were exposed to the test substance at concentrations that closely approximated the intended exposure levels. Thus, the results are a conservative estimate of the effects of the test substance on the test organisms.

The trout LC_{50} value, based upon mean measured concentrations, was 491 mg/L. In addition to a control, nominal test substance concentrations evaluated were 25, 50, 100, 200, 400, and 800 mg/L, and ***all test substance concentrations were greater than the reported water solubility (see*** discussion of water solubility data). The observations on the test solutions described the 400 and 800 mg/L treatments as cloudy at initiation and remained cloudy throughout the duration of the study. Again these observations suggest that at the 400 and 800 mg/L dose concentrations the organisms were exposed to a colloidal suspension or an ultrafine emulsion. There was also a surface film noted initially in treatments greater than and equal to 50 mg/L, which was persistent throughout the exposure.

Since the treatment solutions were prepared individually for each treatment replicate, analytical verification was performed from samples collected from each treatment replicate. The mean analytical recoveries of the non-centrifuged treatment samples at initiation were 90, 94, 91, 87, 9 1, and 85% of the nominal concentrations. The replicate recoveries ranged from 8 1 to 99%. After 96 hours of testing, the mean recoveries in the non-centrifuged treatment samples were 64, 78, 74, 81, 82, and 91% of the nominal and the replicate recoveries ranged from 5 1 to 96%. Even though the analytical recovery for one of the 25 mg/L treatment replicate test chambers fell below 70% of the 0-hour value, the test substance still satisfied the definition of a stable test substance since the mean measured concentrations at this and all other treatment levels remained within 70% of the initial measured values as defined in OPPTS 850.100 (e)(1). There were no residues above the MQL (i.e., 5.85 mg/L) in the control solution samples. Again the mortality (100% in 800 mg/L treatment) and dose related sublethal effects in the 100 (20% effected animals), 200 (60% effected animals) and 400 mg/L (100% effected animals) strongly suggest that there was a significant exposure to the test substance.

Pseudokirchneriella subcapitata EC_{50} (area under the growth curve) and NOEC values (all parameters) based upon mean measured concentrations were 24 mg/L and 11.4 mg/L, respectively. In addition to a control, nominal test substance concentrations evaluated were 1.6, 3.1, 6.3, 13, 25, 50, and 100

mg/L, and *all were greater than the reported water solubility* (see discussion of water solubility data). The solution observations (all normal) indicated that *the test substance may have been dispersed as a microcolloidal suspension or ultrafine emulsion.* Since the treatment solutions were prepared in a single vessel and distributed into replicate test chambers, a single analysis was performed for each treatment at 0, 72, and 96 hours. The samples collected at 72 and 96 hours were centrifuged to eliminate the algae prior to the analytical verification. The analytical recoveries of the test substance treatment solutions at initiation were 95, 103, 87, 99, 90, 96, and 91% of the nominal concentrations. After 72 hours of testing, the recoveries of the test substance treatment solutions after centrifugation were 79, 97, 91, 84, 95, 96, and 94% of the nominal concentrations. After 96 hours of exposure the recoveries for the test substance treatment solutions after centrifugation were 79, 86, 85, 80, 9 1, 93, and 105% of the nominal concentrations. The results from the samples that were centrifuged in the algal study indicate that up to test substance concentrations of 100 mg/L there was no appreciable loss of test substance due to centrifugation. *Therefore, at concentrations greater than the reported water solubility, centrifugation did not remove the microcolloidal suspension or ultrafine emulsion of the test substance from the water column.* While not measured, it was inferred that centrifugation would yield similar results at test substance concentrations of 200,400, and 800 mg/L (see discussion of *D. magna* and trout data), where solutions at these concentrations were cloudy. Substances in microcolloidal suspensions or ultrafine emulsions are considered biologically available to the test organisms.

Even with the possible loss due to the surface *film* in the case of the *D. magna* and trout studies, the recoveries of the mean measured concentrations at test termination for all studies were within 70% of the 0 hour data. According to OPPTS 850.100 (e)(1) mean measured concentrations greater than or equal to 70% of the nominal day zero concentration indicated that the test substance was stable for the study duration. The results from the centrifuged samples analyzed during the algae study clearly indicate that although the test substance may be either a microcolloidal suspension or an ultrafine emulsion, that the analytical measurements represent a conservative measure of the biologically available fraction of the test substance as specified in OPPTS 850.1000 (h)(1). Although the test solution samples for the *D. magna* and trout studies were not centrifuged prior to analysis, the mean measured concentrations approximated the nominal values indicating that the measured concentrations represent the biologically available fraction of the test substance. This assumption is supported further by the biological dose response to the test substance.

In conclusion, the studies conducted for solubility and Log P were done according to USEPA Guideline protocols. For the water solubility study the initial experiment used the shake flask method. ***In ultrapure water, two*** distinct phases were formed with the aqueous layer being cloudy. The results of the initial water solubility experiment was interpreted to indicate that an emulsion had formed in the aqueous layer and thus the column elution method was selected as the appropriate approach to determine the water solubility of the test material. The column elution solubility study indicated that the solubility of the test substance was less than 0.0001 mg/L. For the Log P determination the HPLC chromatographic method was selected. Bracketing standards were used and the measured Log P value equaled 6.2. The water solubility as determined by the column elution method and the Log P determined by the HPLC method are guideline studies with no significant protocol deviations. ***Thus the data generated from these studies are considered entirely valid. The data are consistent, i.e., low water solubility and high Log P.***

Taken on face value there is a discrepancy between the physical chemical parameters, specifically water solubility and Log P and the results of the aquatic toxicity studies. However, taking into consideration the test water, ultra-pure versus "biologically conditioned" (or freshwater algal medium (FWAM)) for the physical chemical and biological studies, respectively, and the potential formation of a fine emulsion or suspension, the data become compatible. All test substance concentrations in the aquatic toxicity studies were greater than the reported water solubility. In the ecotoxicity studies, films and cloudiness were evident in the acute fish and *Daphnia magna* studies at concentrations greater than or equal to 50 or 200 mg/L, respectively. In the algal studies, where the maximum dose was 8 fold less than the maximum in the fish and invertebrate studies there was no visual evidence of emulsion formation, cloudiness or surface film. The lack of appreciable emulsion formation, cloudiness or surface film in the algal study was at test substance concentrations that were comparable to those in the trout and invertebrate studies where cloudiness or surface films were observed. Centrifugation of the algal media did not lead to the formation of multiple phases, which suggests that the test substance is either a mlcrocolloidal suspension or ultrafine emulsion. Similar results following centrifugation would be anticipated at higher test substance concentrations. According to the USEPA Guideline 850.1000 fme emulsions are considered to be biologically available and thus testing with a fine emulsion is valid. It is assumed that the measured

	concentrations represent a conservative measure of the biologically available fraction of the test substance and the response data are valid for regulation of this test substance. Because of the differences in water, ultrapure versus biologically-conditioned (or FWAM), the potential for the formation of ultratine suspensions or emulsions and the lack of phase formation following centrifugation in the algal studies, all experimental results are considered valid and compatible. The Test Plan and Robust Summaries were changed to reflect the use of biologically-conditioned water, and to note that the measured hexaoxatricosane concentrations were the result of micro-colloidal suspensions or ultrafine emulsions.

EPA COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
Vaporpressure. On page 8 of the robust summary, the submitter reports a value of <.00978 hPa (CO.978 Pa) and on page 9 a value of < 9.78 x 10^{-5} Pa. The submitter needs to correct this substantial conversion error and harmonize the units.	The Robust Summaries have been altered to reflect the appropriate value (< 9.78 x 10^{-5} Pa).